IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	Liew, C.C.	Examiner:	Juliet C. Switzer
Serial No.:	10/085,783		
Filed:	Feb. 28, 2002	Group Art Unit:	1634
Titled:	COMPOSITIONS AND METHODS RELATING TO OSTEOARTHRITIS		
		Conf. No.:	5174

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF Hongwei Zhang UNDER 37 C.F.R. §1.132

Sir:

I, **Hongwei Zhang**, Ph.D., hereby declare that:

1. I received a Ph.D. degree from the Institute of Medical Science at the University of Toronto in 2002, and a Master of Science degree from the Department of Immunology at the University of Toronto in 1995. In addition I received my Medical Degree from the University of Medical Sciences in Changchun China in 1989 and practiced as a staff physician for 4 years in Beijing prior to commencing my post graduate studies. I currently hold the positions of Senior Scientist and Scientific Program Leader of Functional Genomics as well as Manager of Research and Development at ChondroGene Inc.

I am one of the inventors of the above-noted U.S. patent application.

I am particularly experienced in the field of osteoarthritis having first starting worked as a Research Associate at the Arthritis Center of Excellence of Toronto Western Hospital, and subsequently receiving a Fellowship from the institute to pursue my PhD studies focusing on the area of osteoarthritis.

genetically-modified human chondrocytes *in vitro*. Osteoarthritis and Cartilage 1998;6:153-160.

Hongwei Zhang, Donna Phang, Ronald M. Laxer, Earl D. Silverman, Sueihua Pan, and Paul J. Doherty. Evolution of the T cell receptor beta repertoire from synovial fluid T cells of patient with juvenile onset rheumatoid arthritis. J. Rheumatol. 1997;24:1396-402.

Petro Lastres, Anihoa Letamendia, **Hongwei Zhang**, Carlos Rius, Nuria Almendro, UIla RAab, Louis A. Lopez, Carmen Langa, Angels Fabra, Michelle Letarte and Carmelo Bernabeu. Endoglin modulates cellular responses to TGF-beta 1. J. Cell Biol. 1996;133:1109-1121.

Hongwei Zhang, Andrew R.E. Shaw, Allan Mak, and Michelle Letarte. Endoglin is a component of the Transforming Growth Factor (TGF)-beta receptor complex of human pre-B leukemic cells. J. Immunol. 1996,156:565-573.

2. I have read the final Office Action mailed June 7, 2005 in the above-referenced patent application.

The Office Action states that Claim 58-73 are rejected under 112 first paragraph, enablement. The rejection first describes the nature of the invention as follows:

> "the nature of the claimed invention requires the knowledge of an association between the gene expression of the ten elected genes and osteoarthritis, or some stage of osteoarthritis as recited in the claims." The practice of the claimed invention for the "diagnosis" of OA or the staging of OA requires the knowledge that not only are genes differentially expressed in OA, but also that this expression is specific to OA or a stage of OA in such a way that one can reliably draw conclusions for the diagnosis of OA based on the gene expression patterns"."

The Office Action asserts that before conclusions can be drawn regarding the diagnosis or staging of OA using the selected genes, that there are unresolved issues including the following:

> "given the small sample size, it is not clear that these data are representative of any population or [of] simply of differences between individuals. No statistical analysis is given. For example, the relative expression is decreased in severe OA versus mild OA but it is increased in normal versus both of these."

"Further, the specification does not provide any controls of individuals with other diseases or disorders so that it is not clear if the differences in expression are specific to OA or are generalized responses to disease, for example"

The Office Action further indicates:

"It is not clear that the test itself of "relative EST frequency" is valid given that the total pool of EST tested in each sample is different. The changes in "relative frequency" could be a result of differences in expression levels of other genes that cause the total number of expressed genes to increase or decrease relative to the gene in question" Almost every single gene that displayed appreciable expression in these libraries did so at different relative levels."

"It is highly unpredictable as to whether any of the apparent differential expression observed in applicants' experiments is specific to OA or to any stage of OA or if it represents some more generalized responses which might be observed in a variety of different conditions"

and

"The data presented for the remaining genes in this group represents very low transcript numbers. A single MAFB EST was detected in fetal and normal samples, none in mild cases and 13 (representing 0.09%) in severe cases. Like B2M, there is not a progression from normal to severe in expression levels, instead the mild case has no expression of this gene. It is not clear how to apply this result to diagnosis of any level of OA, alone or in combination with other genes, as claimed"

3. As a scientist skilled in the area of osteoarthritis and molecular biomarker identification, I submit that the specification provides guidance to enable one of one skill in that art to make and reliably use two or more of the ten elected genes in the claimed methods of diagnosing an individual as having OA.

In our work described in the patent application, we prepared the relevant chondrocyte cDNA libraries by isolating cartilage RNA from two normal individuals (individuals without osteoarthritis), six individuals diagnosed as having mild osteoarthritis, and three individuals diagnosed as having severe osteoarthritis. Diagnosis was performed using the scoring system described by Marshall (1996) *The Journal of Rheumatology, 23* 582-584. RNA isolated from each group of individuals (ie normal, mild or severe)

were pooled and a cDNA library for each pooled group created by synthesizing cDNA from the mRNA and inserting the resulting cDNA into λTripleEx2 vector as described. We isolated and sequenced 57,422 ESTs for the purposes of our analysis. In particular we isolated 17,151 ESTs from the "normal" library, 12,651 ESTs from the "mild" library, and 14,222 ESTs from the severe library.

ESTs are considered by those in the art to represent the gene transcript population of the tissue since ESTs are obtained from the randomly picked cDNA clones of a cDNA library constructed from a tissue. Moreover, the transcript abundance of the original tissue is reflected by its EST frequency in the library. Thus, it is understood in the art that genes which are differentially expressed in a tissue sample from a human individual with a diseased state such as osteoarthritis can be identified by comparing their relative EST frequency levels in normal and diseased tissues. Further, different severities of a disease such as osteoarthritis can also be identified by comparing their relative EST frequency levels in tissues obtained from individuals having varying stages of the disease . This EST-based approach of analyzing transcripts in diseased and normal samples is an accepted scientific approach to studying differential gene expression between these samples (Kumar S, Connor JR, Dodds RA, Halsey W, Van Horn M, Mao J. et al. Osteoarthritis Cartilage. 2001 Oct;9(7):641-53; Dahl et al. The Journal of Pathology 2005 205 (1) 21-28.)

Note that the Office Action suggests that "It is not clear that the test itself of "relative EST frequency" is valid given that the total pool of EST tested in each sample is different". For each gene listed in Figure 6, the number of ESTs isolated from each library for that gene is noted, as well as the frequency (in terms of percentage) of ESTs isolated from each library for that gene relative to the total EST population. The percentage noted is the number of ESTs identified in the library noted for that specific gene divided by the total number of ESTs identified in the library. Thus, for example, for the selected gene B2M noted on line 6 of Figure 6, 88 ESTs corresponding to B2M

were identified from the normal library which contained a total number of 17,151 ESTs. These 88 clones thus represent 0.51% (88/17,151*100) of the total number of ESTs identified in the normal library, which reflects the approximate percentage of B2M related ESTs present in the normal library. Similarly, Figure 6 indicates that 200 ESTs corresponding to B2M were identified from the mild OA library which contained a total of 12,651 ESTs. Thus, these 200 ESTs represent 1.58% (200/12,651*100) of the total number of ESTs identified in the mild OA library, which reflects the approximate percentage of B2M related ESTs present in the mild OA library. Figure 6 shows that 196 ESTs corresponding to B2M were identified in the severe OA library which is 1.38% of the total number of ESTs present in the severe library.

Subsequent to the filing of the current patent application, we continued our work in selecting, sequencing, and annotating ESTs identified from the cDNA libraries disclosed in the specification, and have isolated over 99,000 ESTs; 22,516 ESTs from the fetal library; 24,583 ESTs from the normal library; 25,171 ESTs from the mild library and 27,236 ESTs from the severe library. We have continued our analysis of EST frequency based on these additionally identified ESTs. Attached as Exhibit "A" to this Declaration is a summary of data relevant to the elected genes, namely B2M, TNFAIP6, BCL6, CCNC, IL13RA1, LAMC1, ZFR, MAFB, PER1, PF4, CALM1, and TCTP. Thus the size of our samples of ESTs which have been analyzed has increased, and yet for almost all these elected genes, this additional EST data displays similar patterns of differential gene expression between the libraries as illustrated by the originally disclosed EST data. This data confirms the disclosed EST based differential expression data disclosed in the specification, although, as would be expected the percent frequency in most cases has increased.

The Office Action discusses the MAFB gene and comments "Like B2M, there is not a progression from normal to severe in expression levels, instead the mild case has no expression of this gene." However, it is not

necessary that the degree of differentiated expression of a gene relative to normal be directly or inversely correlated with the degree of severity of the disease for that gene to be a useful biomarker of osteoarthritis. In fact, in many instances, the level of expression of a gene in a sample from an intermediate stage of disease can be higher than that of both an earlier and a later stage of disease. Regardless of whether or not the level of gene expression increases with increasing disease severity, the variation or fluctuation in gene expression as between stages of osteoarthritis is very useful in determining whether an individual has a specific stage of osteoarthritis. As would be understood, it is important in utilizing the elected genes as biomarkers of osteoarthritis, or a stage thereof, that the one compare the expression pattern of a test individual as against the appropriate controls so as to allow the diagnosis OA, or a specific stage thereof.

Microarray Data

Complementary DNA (cDNA) array technology has gained its status by providing the mRNA expression profiling of thousands of genes simultaneously and has allowed us to pursue data for many of the genes identified in Figure 6. To further confirm the utility of many of the biomarkers selected using our EST approach as biomarkers of osteoarthritis, we profiled an additional 18 cartilage samples (7 normal, 8 moderate and 11 severe OA) on our in-house 15k ChondroChip™ constructed using the cDNA clones identified from the four cartilage libraries identified in the current Application. We also profiled an additional set of 60 cartilage samples (10 normal; 23 mild, 7 moderate, 9 marked OA, 11 severe OA) using the Affymetrix® Genechip U133A and U133B arrays. For the Affymetrix® Genechip array, the mean signal from each array was globally scaled to 500 using Affymetrix GCOS software version 1.1.1. Scaled images were imported into GeneSpring (Silicon Genetics; Redwood City, CA).

For our own ChondroChip™, each slide was scanned using an Affymetrix GMS428 confocal microscope laser scanner using the Array Scanner software (Affymetrix, Santa Clara, CA). The scanned images were then analyzed using the ScanAlyze v2.44. The intensity data exported by ScanAlyze was then imported into the GeneSpring (Silicon Genetics, Redwood City, CA) for further analysis.

The Wilcox Mann Whitney test was used to identify differentially expressed genes with a $P<0.05$ when comparing profiles from the osteoarthritic samples with the non osteoarthritic samples.

Results and analysis of Microarray Data

Attached as Exhibit "A" to this Declaration is a summary of the data for either Affymetrix® Genechip array and/or the ChondroChip® array which demonstrates that the ten elected genes are differentially expressed in cartilage from osteoarthritic as compared to non osteoarthritic individuals. Also noted in each instance is the statistical significance (p value) as determined using the Wilcox Mann Whitney test. Multiple p values are shown when more than one probe is used to target the same gene on the microarray platform.

The microarray data shows that all but one of the elected ten genes demonstrated statistically significant differential expression as between individuals having osteoarthritis and individuals not having osteoarthritis. It is significant that the samples tested using the microarray data were not from the same individuals whose samples were used in constructing the cDNA libraries disclosed in the specification. Thus the microarray data confirms the differential expression identified by the EST data for all but one of the elected ten genes. Although in some instances there are discrepancies between the data resulting from the ChondroChip™ or Affymetrix® Genechip analysis (e.g. TCTP and IL13RA1), this does not detract from our overall conclusion of differential expression of all but one of the elected genes with respect to OA. These discrepancies in gene expression data in only two of the elected genes on

these two microarrays may represent technical differences as between the two microarrays, or may be a result of the different compositions of the populations utilized as between the two experiments.

In view of the above, I submit that the specification provides ample guidance to enable one of one skill in that art to practice the claimed methods of diagnosing OA using the ten elected biomarkers.

4. I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that wilful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such wilful false statements may jeopardise the validity of the application or any patent issuing thereon.

Hongwei Zhang, Ph.D. Zhang Hongwei

Date Dec. 07, 2005

Exhibit "A" to Declaration of Hongwei Zhang dated December 7, 2005

Source of Data													
		Gene Name	Accession #	Fetal		Normal		Mild		Severe		Total	OA/ Normal (fold change)
B2M													
Patent Filing 2002	6	beta-2-microglobulin gene (B2M)	AF072907.1	6	0.04%	88	0.51%	200	1.58%	196	1.38%	490	upregulated
Updated EST Data 2004		AF072907.1		7	0.03%	161	0.65%	485	1.93%	415	1.52%	1068	upregulated
Affymetrix Hybridization Data (using Cartilage Samples)													0.749 (downregulated); pvalue 1.76 x 10-6; 0.91 (downregulated); p value1.53 x 10-3
ChondroChip Hybridization Data (using Cartilage)													0.73 (downregulated); pvalue <0.05;
TNFAIP6													
Patent Filing 2002	2654	tumor necrosis factor alpha-induced protein 6 (TNFAIP6)	NM_007115.1	0	0.00%	0	0.00%	1	0.01%	1	0.01%	2	upregulated
Updated EST Data 2004				0	0.00%	0	0.00%	21	0.08%	13	0.05%	34	upregulated
Affymetrix Hybridization Data (using Cartilage Samples)													6.91 (p value 3.19 x 10-6) (Upregulated)
ChondroChip Hybridization Data (using Cartilage)													2.62 p value < 0.05 (upregulated)
BCL6													

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Source of Data													
		Gene Name	Accession #	Fetal		Normal		Mild		Severe		Total	OA/ Normal (fold change)
Patent Filing 2002	2287	BCL6 gene	Z79581.1	0	0.00%	0	0.00%	1	0.01%	0	0.00%	2	downregulated
Updated EST Data 2004				0	0.00%	1	0.00%	1	0.00%	0	0.00%	1	
Affymetrix Hybridization Data (using Cartilage Samples)													0.44 (downregulated); pvalue 2.72 x 10-4
CCNC													
Patent Filing 2002	5670	cyclin C (CCNC)	NM_005190.2	0	0.00%	0	0.00%	1	0.01%	0	0.00%	1	upregulated
Updated EST Data 2004				0	0.00%	1	0.00%	7	0.03%	7	0.03%	15	upregulated
ChondroChip Hybridization Data (using Cartilage)													1.34(upregulated); pvalue 8.90 x 10-3
IL13RA1													
Patent Filing 2002	1366	interleukin 13 receptor alpha 1	NM_001560.1	0	0.00%	0	0.00%	1	0.01%	2	0.01%	3	upregulated
Updated EST Data 2004				0	0.00%	2	0.01%	4	0.02%	4	0.01%	10	upregulated
Affymetrix Hybridization Data (using Cartilage Samples)													1.45 (upregulated) p value 1.74 x 10-2
ChondroChip Hybridization Data (using Cartilage)													0.77 (downregulated) p value 5.4x10-3
LAMC1													

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Source of Data													
		Gene Name	Accession #	Fetal		Normal		Mild		Severe		Total	OA/ Normal (fold change)
Patent Filing 2002	988	laminin, gamm 1 (formerly LAMB2)	NM_002293.2	1	0.01%	4	0.02%	0	0.00%	0	0.00%	5	downregulated
Updated EST Data 2004				2	0.01%	7	0.03%	5	0.02%	4	0.01%	16	downregulated
ChondroChip Hybridization Data (using Cartilage)													0.81 (downregulated); pvalue 1.21x10-2
ZFR													
Patent Filing 2002	4595	zinc finger RNA binding protein (Zfr)	AF071059.1	1	0.01%	0	0.00%	0	0.00%	0	0.00%	1	
Updated EST Data 2004				1	0.00%	4	0.02%	7	0.03%	11	0.04%	22	upregulated
Affymetrix Hybridization Data (using Cartilage Samples)													0.81 (downregulated); pvalue 4.01 x 10-5
MAFB													
Patent Filing 2002	316	MAFB/Kreisler basic region/leucine zipper transCRiption factor (MAFB)	AF134157.1	1	0.01%	1	0.01%	0	0.00%	13	0.09%	15	upregulated
Updated EST Data 2004				2	0.01%	1	0.00%	0	0.00%	29	0.11%	30	upregulated
PER1													
	3596	PER1 gene (=Rigui (RIGUI))	AF102137.1	0	0.00%	1	0.01%	0	0.00%	0	0.00%	1	downregulated

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Source of Data													
		Gene Name	Accession #	Fetal		Normal		Mild		Severe		Total	OA/ Normal (fold change)
				1	0.00%	2	0.01%	0	0.00%	0	0.00%	3	downregulated
Affymetrix Hybridization Data (using Cartilage Samples)													0.36 (downregulated) p value 7.86 x 10-7 ; 0.43 (downregulated) p value 3.9 x 10-5
PF4													
Patent Filing 2002	1304	Krueppel-related DNA-binding protein (PF4)	M61866	0	0.00%	1	0.01%	1	0.01%	1	0.01%	3	
CALM1													
Patent Filing 2002	29	calmodulin 1 (phosphorylase kinase, delta) (CALM1)	NM_006888.1	7	0.05%	23	0.13%	31	0.25%	46	0.32%	107	upregulated
Updated EST Data 2004				5	0.02%	40	0.16%	95	0.38%	130	0.48%	270	upregulated
Affymetrix Hybridization Data (using Cartilage Samples)													1.52 (upregulated); pvalue 7.0 x 10-4; 1.71 (upregulated); p value 2.98x 10-4; 2.62 (upregulated) p value 6.23 x 10-6
ChondroChip Hybridization Data (using Cartilage)													2.74 (upregulated) p value 1.28 x 10-7; 1.61 (upregulated) p value 3.01 x 10-5
TCTP													

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Source of Data													
		Gene Name	Accession #	Fetal		Normal		Mild		Severe		Total	OA/ Normal (fold change)
Patent Filing 2002	47	translationally controlled tumor protein (TCTP)	X16064	23	0.17%	14	0.08%	17	0.13%	28	0.20%	82	upregulated
Updated EST Data 2004				67	0.30%	71	0.29%	70	0.28%	89	0.33%	297	upregulated
Affymetrix Hybridization Data (using Cartilage Samples)													1.02 (upregulated) p value 1.78 x 10-2; 0.79 (downregulated) p value 1.16x10-2